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Clinical and Laboratory Evaluation of Cefamandole in the Therapy of *Haemophilus* spp. Bronchopulmonary Infections

DENNIS G. DELGADO, CARMEN J. BRAU, C. GLENN COBBS, AND WILLIAM E. DISMUKES*
Division of Infectious Diseases, Department of Medicine, The University of Alabama School of Medicine, Birmingham, Alabama 35294

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A prospective, randomized, single-blind comparison of parenteral cefamandole and ampicillin was conducted in 27 hospitalized adult patients with pneumonia or purulent tracheobronchitis due to *Haemophilus* spp. Patients received either parenteral cefamandole or ampicillin in a dose of 1 g every 6 h. Cefamandole was as effective and safe as ampicillin. Of the 14 patients treated with cefamandole, 13 were considered cured, as were 12 of the 13 treated with ampicillin. One patient in each treatment group improved clinically but did not clear his sputum of *Haemophilus* spp. One patient treated with cefamandole had a recurrence of *Haemophilus* spp. bronchitis 9 days after cure. Adverse effects were more common in the cefamandole-treated group (50% versus 15%), but were mild and did not require discontinuation of therapy in any patient. The in vitro susceptibilities of 64 clinical isolates of *Haemophilus* spp. to 10 antibiotics were determined. Cefamandole was the most active of the cephalosporin-cephamycin antibiotics tested, inhibiting 98% of 61 non-beta-lactamase-producing isolates at 2 μg/ml and 100% at 4 μg/ml. Cefamandole inhibited the three ampicillin-resistant isolates at 2 μg/ml or less. Cephapirin, cefoxitin, and cephalothin were the next most active, whereas cefazolin and cephradine were the least active.

Ampicillin has generally been regarded as the antimicrobial agent of choice for *Haemophilus influenzae* bronchopulmonary infections (13). However, there have been recent reports of ampicillin-resistant *H. influenzae* strains, particularly type B (10, 12, 20, 23). Recently, there have also been sporadic reports of resistance to chloramphenicol and tetracycline (14, 23), the usual alternative drugs. Cefamandole, a new cephalosporin with a broad spectrum of activity, has been shown to be effective against various infectious disorders caused by *H. influenzae* (2, 18). The purpose of this study was to prospectively compare the efficacy and toxicity of cefamandole and ampicillin in the therapy of *Haemophilus* spp. bronchopulmonary infections and to determine the in vitro susceptibility of 64 clinical isolates of *Haemophilus* spp. to 10 antimicrobial agents.

MATERIALS AND METHODS

Clinical study. Adult patients hospitalized on the medical services of the Birmingham Veterans Administration Hospital or University Hospital were evaluated for entry into this study. Initial evaluation included a history, physical examination, chest roentgenogram, and sputum examination. Criteria for admission into the study were (i) clinical evidence of acute purulent tracheobronchitis plus a sputum Gram stain consistent with *Haemophilus* spp. infection, or (ii) clinical and chest roentgenographic evidence of pneumonia plus a sputum Gram stain consistent with *Haemophilus* spp. infection, or (iii) culture-proven *Haemophilus* spp. infection which had not responded to initial antibiotic therapy. A *Haemophilus* spp. was presumed to be the etiological agent in either acute purulent tracheobronchitis or pneumonia when the sputum Gram stain showed a predominance of small pleomorphic gram-negative coccobacilli in the presence of polymorphonuclear leukocytes or alveolar macrophages. Patients were not eligible for entry if any one of the following was present: (i) successful or suppressive antimicrobial therapy within 3 days preceding the initial (pretherapy) bacteriological evaluation; (ii) concomitant infection that would interfere with evaluation of drug efficacy; (iii) history of allergy of the immediate type to penicillins or cephalosporins; (iv) pregnancy; and (v) significant renal insufficiency (serum creatinine > 3.0 mg/100 ml).

Thirty-six patients met the criteria for entry, and informed consent was obtained from each. Randomization of patients was carried out using a randomization table (provided by Eli Lilly and Co.) which permitted no more than two successive patients being assigned to the same drug. Patients were randomized in a single-blind fashion to receive either cefamandole (1 g intravenously [i.v.] or intramuscularly every 6 h) or ampicillin (1 g i.v. or intramuscularly every 6 h). The antibiotics when given intravenously were diluted in 100 ml of D_5W or saline and given over 15 to 30 min. Pretreatment studies on each patient included sputum and blood cultures, leukocyte count with dif-

ferential, platelet estimation, Coombs test, blood urea nitrogen, creatinine, serum glutamic oxalacetic transaminase or serum glutamic pyruvic transaminase, total bilirubin, alkaline phosphatase, and urinalysis. These tests were repeated at regular intervals throughout the treatment period and at the conclusion of therapy.

Either expectorated sputum specimens, transtracheal aspirates, or bronchial washings were transported to the microbiology lab within 1 to 2 h after collection. Sputum samples were immediately streaked on blood, chocolate, and Levinthal agar, prepared with washed human erythrocytes (21, 22), and incubated in CO_2 at 37°C. Samples were also inoculated on eosin-methylene blue agar and incubated at 37°C. Cultures were examined at 24 and 48 h. *Haemophilus* spp. were identified by methods described below.

Patients were considered evaluable if all the following criteria were met: (i) the initial sputum culture grew moderate or abundant *Haemophilus* spp. and no other pathogen; (ii) the *Haemophilus* spp. isolate was susceptible to the study drug; (iii) a minimum of 5 days of antimicrobial therapy was completed; and (iv) there was protocol adherence during therapy.

A cure was defined as: (i) a symptomatic response including a diminution in the severity of symptoms and signs and a decrease in the leukocyte count; (ii) a bacteriological response with elimination of the pathogen from the sputum; and (iii) improvement in the chest roentgenogram in cases of pneumonia. A clinical response with bacteriological persistence met all the criteria for cure except that the sputum was not cleared of the pathogen. A recurrence was defined as symptomatic and radiographic deterioration after a documented cure, with the reisolation of the initial pathogen. A superinfection was defined as the development of clinical and radiographic deterioration in a patient receiving therapy for pneumonia associated with the isolation of a pathogen that was not originally present.

Bacteriology. We examined 64 *Haemophilus* spp. isolates by agar dilution for susceptibility to 10 antimicrobial agents. In addition, disk susceptibility to cefamandole and cefoxitin was also examined in 60 isolates. All isolates were obtained from sputum or bronchial washings of patients at the University of Alabama Medical Center and the Mobile General Hospital (kindly supplied by James Bonner). Twenty-seven isolates were from our evaluable study patients, and 37 were from nonstudy patients. *Haemophilus* spp. were identified by X and V growth characteristics, and isolates were typed with *H. influenzae* antisera (Difco Laboratories, Detroit, Mich.). Beta-lactamase production was determined by the use of chromogenic cephalosporin substrate (Glaxo compound 87/312) as originally described by O'Callaghan et al. (16). All of the nonstudy isolates and 20 of the 27 clinical study isolates were *H. influenzae*. The remaining clinical study isolates were *H. aphrophilus* (four) and *H. parainfluenzae* (three). There were three type B isolates: two from clinical study patients, and one from a nonstudy patient. The remainder were nontypable. There were three ampicillin-resistant, nontypable isolates, all from nonstudy patients. Isolates were stored in skim milk at −60°C.

Agar dilution studies. Minimum inhibitory concentrations (MICs) were determined by the agar dilution method as described by Barry (3). Strains were transferred to fresh chocolate agar plates the day before testing. After overnight incubation in CO_2, five to six colonies were selected and suspended in Levinthal broth. The bacterial suspension was adjusted to a McFarland 0.5 standard and diluted 1:100, resulting in a concentration of 10^6 colony-forming units per ml. Antibiotic dilutions were prepared and incorporated into Levinthal agar poured in 100-mm petri dishes. Plates were used within 24 h. An inoculum of approximately 10^3 to 10^4 colony-forming units was delivered by a Steers replicator (19) to 5-mm-diameter areas on the agar plates. The plates were incubated in CO_2 at 37°C for 18 to 24 h. The MIC was defined as the lowest concentration of antibiotic able to completely inhibit growth.

Disk susceptibility testing. Tests were performed using Levinthal agar and inocula of 10^8 colony-forming units from 18-h broth cultures. Cefamandole (Eli Lilly and Co.) and cefoxitin (Merck, Sharp and Dohme) 30-μg disks were used. Zone diameters were measured with a Fisher-Lilly zone reader.

Serum bactericidal activity. The serum bactericidal activity was determined in sera from eight patients receiving cefamandole and six patients receiving ampicillin according to the method described by Barry and Sabath (4). Blood was obtained 60 to 90 min after the i.v. infusion of antibiotics.

Cefamandole levels. Serum concentrations of cefamandole were determined by an agar well diffusion method with *Bacillus subtilis* (ATCC 6633) as the test organism (5). A 1:1,000 dilution of the spore suspension of this bacterium was prepared using antibiotic medium no. 1 (BBL) Microbiology Systems, and 25-ml portions were placed in 150-mm plates. Wells (approximately 2.4 mm in diameter) were filled with approximately 5 μl of each sample. The cefamandole standards were prepared with pooled human serum to achieve final concentrations of 80, 40, 20, 10, 5, 2.5, 1.2, and 0.6 μg/ml. The plates were allowed to refrigerate for 4 h at 4°C and then were incubated overnight at 37°C. The zones of inhibition were measured on a Fisher-Lilly zone reader, and the antibiotic concentrations were determined from a standard curve. All assays were performed in duplicate.

RESULTS

Clinical study. Twenty-seven of the 36 patients randomized were considered evaluable. Of the nine patients who were not evaluable, there were six patients in whom *Haemophilus* spp. were not isolated from the sputum. Among the other three inevaluable patients, one had a cardiopulmonary arrest on day 4 of therapy and expired 2 days later; one had multiple aerobic gram-negative bacilli isolated from sputum in addition to *H. influenzae* and required the use of concomitant antibiotics; and the third was non-protocol adherent.

The demographic and clinical data are depicted in Table 1. Chronic obstructive pulmonary disease was the most common underlying

disease and was present in 23 of our 27 evaluable patients. Fourteen patients were treated with cefamandole; 10 had purulent tracheobronchitis and 4 had pneumonia. Of the 13 patients treated with ampicillin, 10 had pneumonia and 3 had purulent tracheobronchitis. The mean initial temperature and leukocyte count for the cefamandole group were 99.6°F (ca. 37.6°C) and 11,900, respectively, and for the ampicillin group they were 100.6°F (ca. 38.1°C) and 12,100, respectively. *Haemophilus* spp. bacteremia was not documented in any patient.

All patients were treated with a minimum of 5 days of parenteral therapy with either cefamandole or ampicillin, except for one patient who received only 3 days of parenteral ampicillin followed by 10 days of oral ampicillin. In the ampicillin treatment group, there were three patients who received a concomitant antibiotic during the first 24 to 48 h of therapy while results of sputum cultures were pending.

Of the 27 evaluable patients with pneumonia or purulent tracheobronchitis due to *Haemophilus* spp., 12 of 14 treated with cefamandole and 12 of 13 treated with ampicillin were cured. One patient in each treatment group was considered to have had a clinical response with bacteriological persistence since he did not clear his sputum of *Haemophilus* spp. during therapy. One patient treated with cefamandole was considered cured, but 9 days after completion of therapy he had a recurrence of *Haemophilus* spp. bronchitis.

Seven of the 14 patients in the cefamandole group and 2 of the 13 patients in the ampicillin group experienced adverse effects. Four of the 14 patients who received i.v. cefamandole developed mild phlebitis at the infusion site; three of these four had had an indwelling venous catheter for more than 48 h at the time the phlebitis was detected. Both patients who received intramuscular cefamandole (after initial i.v. therapy) experienced mild to moderate pain at the injection site. Three patients in the cefamandole group and one patient in the ampicillin group experienced transient diarrhea during therapy, but this could not be directly attributable to the study drugs. Two patients receiving ampicillin had abnormal liver function tests on admission, which were thought to be due to alcohol abuse. During therapy their liver function tests gradually worsened but then returned toward normal by the completion of therapy. Ampicillin was not judged to be responsible for these alterations.

Cefamandole levels and serum bactericidal activity. The mean serum concentration of cefamandole 60 to 90 min after i.v. infusion of 1 g of cefamandole in eight patients was 9.3 μg/ml (range 1.0 to 37.0 μg/ml), and the geometric mean serum bactericidal activity was 1:13 (range < 1:2 to 1:256). In six patients receiving a 1-g dose of i.v. ampicillin the geometric mean serum bactericidal activity 60 to 90 min postinfusion was 1:25 (range 1:8 to 1:256).

TABLE 1. *Clinical data on 27 patients with acute bronchopulmonary infections due to Haemophilus spp., treated with cefamandole or ampicillin*

Determination	Cefamandole	Ampicillin
No. of patients	14	13
Mean age (years)	58 (25–83)	68 (26–88)
Sex (M/F)	13/1	12/1
Underlying disease[a]		
COPD	12	11
CHF	2	4
Alcoholism	2	2
Cancer of lung		2
S/P splenectomy	3	
None		1
Type of infection		
Purulent tracheobronchitis	10	3
Pneumonia	4	10
Mean days of therapy	10.7 (5–14)	11 (7–15)
Outcome		
Cure[b]	12	12
Clinical response with bacteriological persistence	1	1
Cure followed by recurrence	1	
Adverse effects		
None	7	11
Phlebitis (no. presenting/no. receiving i.v. drug)	4/14	1/13
Pain at injection site (no. presenting/no. receiving i.m. drug)	2/2	0/2
Superinfection	1	1

[a] COPD, Chronic obstructive pulmonary disease; CHF, congestive heart failure.
[b] As defined in the text.

Agar dilution studies. The agar dilution susceptibilities of 61 non-beta-lactamase-producing isolates of *Haemophilus* spp. to 10 antimicrobial agents are shown in Table 2. Ampicillin was the most active of the 10 antibiotics tested, and all strains were inhibited by 0.5 μg/ml or less. Chloramphenicol was the next most active; 98% of the strains were inhibited by 0.5 μg/ml. A concentration of 1 μg of tetracycline per ml inhibited 93% of the strains. Cefamandole was the most active of the cephalosporins or cephamycins tested, with 2 μg/ml inhibiting 98% of the strains and 4 μg/ml inhibiting 100% of strains. Cephapirin at 4 μg/ml inhibited 95% of the isolates, and cefoxitin inhibited 80% of the strains at 4 μg/ml and 90% at 8 μg/ml. Cephalothin at 4 μg/ml inhibited 57% of the isolates

TABLE 2. *Susceptibility of 61 non-beta-lactamase-producing isolates of Haemophilus spp. to 10 antimicrobial agents*

Antimicrobial agent	Cumulative % of strains inhibited at concn (μg/ml) of agent[a]											MIC (μg/ml) active against:	
	≤0.12	0.25	0.5	1	2	4	8	16	32	64	≥128	50% of isolates	90% of isolates
Ampicillin	3	67	100									0.25	0.5
Chloramphenicol	5	21	98	98	100							0.50	0.5
Tetracycline	2	54	82	93	97	100						0.25	1
Cefamandole	3	20	77	89	98	100						0.5	2
Gentamicin	2	3	5	16	61	97	98	98	100			2	4
Cephapirin	2	3	11	18	59	95	100					2	4
Cefoxitin		2	2	3	13	80	90	98	100			4	8
Cephalothin	2	2	5	20	43	57	84	100				4	16
Cefazolin	2	2	3	3	31	39	64	95	100			8	16
Cephradine						3	3	36	69	92	100	32	64

[a] Isolates tested by the agar dilution method.

and 84% at 8 μg/ml. Cefazolin and cephradine were the least active, with 95 and 92% of the strains inhibited at 16 and 64 μg/ml, respectively. Gentamicin at 4 μg/ml inhibited 97% of isolates.

There were three ampicillin-resistant (MIC ≥ 8 μg/ml) beta-lactamase-producing isolates tested against the above antibiotics. Chloramphenicol and tetracycline inhibited these three isolates at 0.5 and 1.0 μg/ml, respectively, whereas cefamandole was again the most active of the cephalosporin-cephamycin drugs, inhibiting these strains at 2 μg/ml or less. The MICs of the remainder of the antibiotics tested were 4 μg/ml or greater against these three beta-lactamase-producing strains.

Disk susceptibility testing. The mean zone of inhibition of 60 cefamandole-susceptible (MIC ≤ 4 μg/ml) *Haemophilus* spp. isolates tested against the 30-μg cefamandole disk was 30 mm (range 24 to 43 mm). Forty-five of these isolates had MICs of 0.25 to 0.5 μg/ml and a mean zone size of 30 mm. Fifteen isolates had MICs of 1 to 4 μg/ml and a mean zone size of 29 mm. The mean zone of inhibition of 59 cefoxitin-susceptible (MIC ≤ 16 μg/ml) *Haemophilus* spp. isolates tested against the 30-μg cefoxitin disk was 27 mm (range 20 to 36 mm). Forty-eight of these isolates had MICs of 1 to 4 μg/ml and a mean zone size of 27 mm. Eleven isolates had MICs of 8 to 16 μg/ml and a mean zone size of 26 mm. One cefoxitin-resistant isolate (MIC = 32 μg/ml) had a zone of inhibition of 16 mm.

DISCUSSION

Recent clinical studies have shown cefamandole to be efficacious and safe in the therapy of various gram-positive and gram-negative infections including those due to *H. influenzae*. Minor et al. (15) treated 17 patients with gram-negative pneumonias with cefamandole. Six of the 17 pneumonias were due to *H. influenzae*. Thirteen of the 17 total and all 6 patients with *H. influenzae* responded satisfactorily without any major untoward effects. Perkins et al. (17) treated 22 adult patients with acute respiratory infections with cefamandole. Four were pneumonias due to *Haemophilus* spp.; six other patients with pneumonia, bronchitis, and lung abscesses had *Haemophilus* spp. isolated as part of a mixed flora. All 22 had a satisfactory clinical response.

In the present study cefamandole and ampicillin appeared equally effective for the treatment of bronchopulmonary infections due to *Haemophilus* spp. Although randomization resulted in cefamandole being used primarily in patients with purulent tracheobronchitis (10 of 14 patients), and ampicillin being used primarily in patients with pneumonia (10 of 13 patients), in our clinical judgement the two treatment groups were roughly comparable. Most of the patients with pneumonia had the bronchopneumonic type, and their clinical illnesses, as judged by mean initial temperature, mean initial leukocyte count, and duration of therapy, were similar to the illnesses caused by tracheobronchitis. The incidence of adverse effects was greater in the cefamandole-treated group (50%) than in the ampicillin-treated group (15%), but these effects were mild and did not require alteration or discontinuation of therapy in any patient.

Since bacteremia was not identified in any of our patients, the diagnosis of acute purulent tracheobronchitis or pneumonia due to *Haemophilus* spp. was based on the results of sputum Gram stains and sputum cultures. Although the significance of non-encapsulated *Haemophilus* spp. in the nonbacteremic forms of respiratory

infections has been questioned, recent studies support their role as pathogens (6, 7).

Azimi (1) reported the susceptibility of 136 blood and cerebrospinal fluid isolates of *H. influenzae* to cefamandole. One hundred thirty-two of these isolates were ampicillin susceptible. Cefamandole inhibited all of these ampicillin-susceptible isolates at 5 μg/ml or less. The results of our study concur with these data, namely that cefamandole at 4 μg/ml inhibited 100% of 61 non-beta-lactamase-producing isolates.

In vitro susceptibility studies by Rodriguez et al. (18) revealed that 95% of 87 ampicillin-resistant strains of *H. influenzae* were inhibited by less than 0.78 μg of cefamandole per ml. Similarly, Jorgensen and Alexander (11) showed that 84% of 32 ampicillin-resistant isolates were inhibited by 2 μg of cefamandole per ml. In the present study cefamandole inhibited the three beta-lactamase-producing isolates at 2 μg/ml or less. This activity of cefamandole against ampicillin-resistant strains of *H. influenzae* correlates with the drug's relative resistance to inactivation by beta-lactamases (8).

The mean serum level of 9.3 μg/ml obtained 60 to 90 min after a 1-g i.v. dose of cefamandole is consistent with the previously reported pharmacokinetics of cefamandole. Griffith et al. (9) reported a mean peak level of 139 μg/ml at 10 min, declining to 15 μg/ml at 1 h and then to 4.9 μg/ml by 2 h after a 1-g i.v. dose of cefamandole. The higher serum bactericidal titers with ampicillin (mean = 1:25) in comparison to cefamandole (mean = 1:13) can in part be explained by the lower MICs of ampicillin (0.5 μg/ml inhibited 90% of isolates) versus cefamandole (2.0 μg/ml inhibited 90% of isolates) (Table 2).

The results of our study suggest that cefamandole is an effective parenteral cephalosporin for the treatment of purulent tracheobronchitis or pneumonia due to *Haemophilus* spp., especially in patients with chronic obstructive pulmonary disease. Cefamandole should be considered for the therapy of patients who have disease due to ampicillin-resistant *Haemophilus* spp., for patients who are allergic to penicillin, or for patients who are high risk for therapy with chloramphenicol or tetracycline. Cefamandole also has the advantage of providing broad-spectrum coverage against *Streptococcus pneumoniae*, *Streptococcus* spp., *Staphylococcus aureus*, and other gram-negative aerobic bacilli, e.g., *Escherichia coli*, *Proteus* spp., and *Enterobacter* spp., which may also be pathogenic in patients with chronic obstructive pulmonary disease.

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